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Microbiological Transformations of Nabilone, a Synthetic Cannabinoid

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A screening program was conducted to find microorganisms that modify the synthetic cannabinoid nabilone. After purification, the products from three cultures were analyzed by spectral methods to determine their chemical structures. An optically active 9S-hydroxy-6aR,10aR-*trans* cannabinoid was isolated from a culture of an unidentified soil bacterium designated A24007. From *Bacillus cereus* cultures were isolated a 9S,6'-dihydroxy-6aR,10aR-*trans* cannabinoid, a 9S-hydroxy-6'-keto-6aR,10aR-*trans* cannabinoid, a 9-keto-6'-hydroxy-6aS,10aS-*trans* cannabinoid, and a 6',9-diketo-6aS,10aS-*trans* cannabinoid. All of these products were optically active, as was a 9S-hydroxy-6aS,10aS-*trans* cannabinoid also isolated from *B. cereus* cultures. A series of acidic products were isolated from cultures of *Nocardia salmonicolor*. All of these products contained a carboxylic acid group at the terminal end of three-position alkyl side chains having varying numbers of carbon atoms. Two of the acidic products contained a 9-keto group, whereas all other carboxylic acid products were 9-hydroxy cannabinoids. The array of products obtained from incubation of nabilone indicates the usefulness of microbial transformations in the preparation of new cannabinoids.

Marihuana has been reported to produce a wide variety of pharmacological effects in humans (7). Some of these effects, such as tachycardia (13, 14), are undesirable for many therapeutic indications. Other effects reported for marihuana or the isolated natural product Δ^9-tetrahydrocannabinol (Δ^9-THC) are clearly of clinical significance, including (i) the control of nausea and vomiting in cancer chemotherapy patients (20), (ii) the lowering of ocular pressures in glaucoma patients (10, 11), and (iii) the alleviation of pain (15).

In attempts to minimize the undesirable effects while enhancing the desired clinical effects, new cannabinoids have been prepared by de novo synthesis (16) and by chemical and microbiological conversions (1, 5, 6, 8, 17, 18, 22) of both naturally occurring and synthetic cannabinoids. Previous studies (9) in our laboratories demonstrated that 4' hydroxylation of the *n*-pentyl side chain of $\Delta^{6a,10a}$-THC occurred in cultures of *Bacillus cereus*.

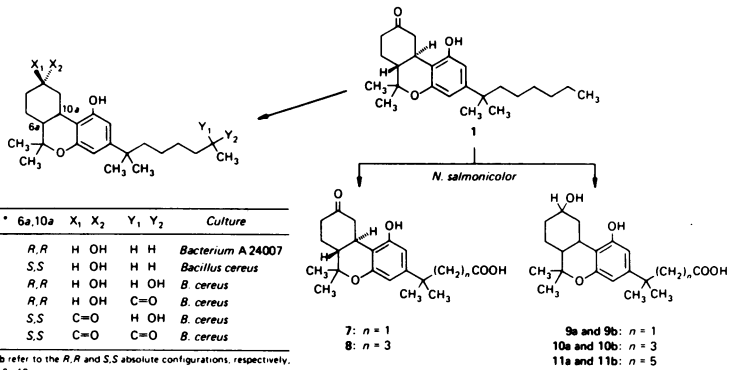
The present studies were undertaken to examine the action of microorganisms on the synthetic cannabinoid nabilone. (See Fig. 1 for chemical structures.) Nabilone was chosen as a substrate for the following reasons: (i) unlike $\Delta^{6a,10a}$-THC and all of the naturally occurring cannabinoids, nabilone possesses a 1,1-dimethylheptyl three-position side chain, and (ii) nabilone is currently undergoing clinical evaluation as an antiemetic agent in cancer chemotherapy patients (12), as an ocular pressure-reducing agent in glaucoma patients (23), and as an antianxiety agent (R. L. Ilaria and W. E. Fann, Fed. Proc. 37:619, 1978).

It was our hope that some microbial conversion product of nabilone might possess new or improved pharmacological properties, might be useful as an intermediate for further synthetic modification, or might prove to be identical to a human metabolite whose chemical structure was in doubt.

MATERIALS AND METHODS

Microorganisms. A screening program was conducted to find microorganisms that modify nabilone (Fig. 1, compound 1). The screening procedure, described in a previous paper (9), used microorganisms from the Lilly Research Laboratories culture collection. Some of the microorganisms were randomly selected, unidentified soil isolates. The microorganisms that produced the transformation products reported in this paper are as follows: (i) a strain of *B. cereus* NRRL B8172, designated culture A36659, (ii) an unidentified gram-negative, rod-shaped microorganism, designated culture A24007, and (iii) a strain of *Nocardia salmonicolor* ATCC 19149, designated culture A36689.

Production and isolation of compound 2a. Culture A24007, a bacterium isolated from soil, was maintained on Trypticase soy agar slants (BBL Microbiology Systems, Cockeysville, Md.). Loop inocula from the slants were transferred to 500-ml Erlenmeyer



Entry*	6a,10a	X_1 X_2	Y_1 Y_2	Culture
2a	R,R	H OH	H H	*Bacterium* A 24007
2b	S,S	H OH	H H	*Bacillus cereus*
3a	R,R	H OH	H OH	*B. cereus*
4a	R,R	H OH	C=O	*B. cereus*
5b	S,S	C=O	H OH	*B. cereus*
6b	S,S	C=O	C=O	*B. cereus*

*a and b refer to the R,R and S,S absolute configurations, respectively, of the 6a,10a ring juncture

FIG. 1. *Microbiological transformation products of nabilone (compound 1).*

flasks containing 100 ml of Trypticase soy broth. The flasks were incubated at 30°C on a rotary shaker operating at 250 rpm with a 2.5-inch (ca. 6.3-cm) stroke. After 24 h of cultivation, 50 mg of nabilone (compound 1) in 1 ml of ethanol was added to each flask, and the incubation was continued for an additional 72 h. The 9-hydroxy transformation product (compound 2a) was then recovered by extracting the cultures three times with ethyl acetate, using 0.5 volume of solvent per volume of culture. The extracts were combined, washed twice with 0.1 volume of water, dried over Na_2SO_4, and concentrated in vacuo to dryness. Compound 2a (Fig. 1) was purified from the dried extract by preparative thin-layer chromatography, using Silica Gel 60 F_{254} plates (E. Merck, Darmstadt, Germany) The plates were developed in a benzene–ethyl acetate (1:1) solvent system, and the product R_f was 0.25.

Production and isolation of compounds 2b, 3a, 4a, 5b, and 6b. Cells of *B. cereus* were grown on glucose in a defined mineral salts medium and then resuspended in 0.1 M phosphate buffer (9). Nabilone (80 mg) was dissolved in 1 ml of ethanol and added to 125 ml of buffer–cell suspension in 500-ml Erlenmeyer flasks. The flasks were incubated at 30°C on a rotary shaker for 7 days. The transformation products were then recovered by extracting the flask contents four times with ethyl acetate, using 0.5 volume of solvent per volume of culture. The extracts were combined, washed twice with 0.1 volume of water, dried over Na_2SO_4, and concentrated to dryness in vacuo.

The transformation products were partially purified by chromatographing the dried ethyl acetate extracts on a Silica Gel 60 prepack column (size B; E. Merck). The column was equilibrated with chloroform, and 0.5 g of extract, dissolved in 1.0 ml of chloroform, was injected onto the column. The products were eluted with a stepwise solvent gradient containing increasing amounts of ethyl acetate in chloroform. Column fractions containing the products were dried in vacuo and then further purified by preparative thin-layer chromatography on Silica Gel 60 F_{254} plates, using a benzene–ethyl acetate (1:1) solvent mixture.

Production and isolation of acidic nabilone transformation products. Stock cultures of *N. salmonicolor* were maintained on ISP medium no. 2 (Difco Laboratories, Detroit, Mich.) agar slants. A loop inoculum from a slant was transferred to 100 ml of P-1 mineral salts medium (2) in a 500-ml Erlenmeyer flask. The medium contained 1% (vol/vol) hexadecane as the sole carbon source. The inoculated flask was incubated at 30°C on a rotary shaker (250 rpm; 2.5-inch [ca. 6.3-cm] stroke) for 48 h. Portions (4 ml) of this culture were transferred to 200-ml volumes of P-1 medium (1% hexadecane) in 1,000-ml Erlenmeyer flasks. The flasks were incubated at 30°C on the rotary shaker for 24 h, and then nabilone was added. Before addition, nabilone was mixed with polyvinylpyrrolidone (1:10, wt/wt) and then dissolved in chloroform. The chloroform was removed in vacuo, and the resulting solid residue was suspended in sterile water. A portion of this suspension was added to each flask to give a final concentration of 0.25 mg of nabilone and 2.5 mg of polyvinylpyrrolidone per 1 ml of culture. After nabilone addition, the cultures were incubated for 10 days and then extracted to recover the transformation products.

Before extraction, the cultures were pooled, and the pH was adjusted to 4.0 with concentrated HCl. The pooled whole-culture broth was extracted three times with 0.5 volume of ethyl acetate. The ethyl acetate extracts were combined, washed twice with dilute HCl, and then dried over Na_2SO_4. The extracts were then concentrated in vacuo to one-quarter of the initial volume and extracted twice with 0.5 volume of 0.05 N NaOH and once with 0.1 N NaOH. The NaOH extracts were combined, the pH was adjusted to 5.0 with HCl, and the solutions were extracted four times with 0.33 volume of ethyl acetate. These extracts were combined, dried over Na_2SO_4, and concentrated in vacuo to dryness. The acidic products were isolated from the dried extracts by high-performance liquid chromatography on Porasil A(60) (Waters Associates, Inc., Milford, Mass.) packed in a stainless steel column (6 feet [ca. 1.83 m] by 11-mm ID) equilibrated in chloroform. The extract was dissolved in a small quantity of ethyl acetate and filtered (paper no. 588; Schleicher & Schuell Co., Keene, N.H.) to remove insoluble material. The filtrate was injected into the column, and the acidic products were eluted with a stepwise solvent gradient that contained chloroform with increasing concentrations of ethyl acetate.

The acidic products that eluted from the column were further purified by preparative thin-layer chromatography on Silica Gel $60F_{254}$ plates, using an ethyl acetate–benzene–hexane–methanol (8:1.5:1.0:0.5) solvent system.

Control experiments. To ensure that the compounds isolated from the cultures were enzymatic transformation products, control flasks were prepared. Control and experimental flasks were prepared by similar procedures, except that the control flasks were autoclaved for 48 h after inoculation (two sterilization cycles at 121°C for 30 min). Control flasks were also prepared by not adding nabilone to viable cultures. Control and experimental flasks were incubated, extracted, and analyzed by similar procedures.

Physical and chemical analyses. High-resolution mass spectra were obtained with a Varian Mat 731 spectrometer (Varian Associates, Palo Alto, Calif.). Proton magnetic resonance (PMR) spectra were measured with Varian Associates HA-100 and HA-200 spectrometers; chemical shifts are expressed in parts per million (δ) downfield from the internal standard tetramethylsilane. Infrared (IR) spectra were obtained by using a model 457 diffraction grating spectrophotometer (The Perkin-Elmer Corp., Norwalk, Conn.). Ultraviolet (UV) spectra were measured with a Cary 15 spectrophotometer (Varian Associates). Optical rotations were determined with a Perkin-Elmer 241 polarimeter.

Nabilone (compound 1). The substrate nabilone (Fig. 1, structure 1) was prepared by the procedure of Archer et al. (4). The compound has the following physical and chemical characteristics: mp, 159 to 160°C; UV (ethanol) λ_{max}, 207 and 280 nm (ϵ = 47,000,250); IR ($CHCl_3$), 5.85 μm (C=O); ^1H nuclear magnetic resonance (NMR) ($CDCl_3$), δ 7.75 (s, 1H, exchanges with D_2O), 6.34,6.35 (2d, 2H, J = 2Hz, H_2 and H_4), 4.15 (d, 1H, J = 14.3 Hz, H_{10}), 3.08-0.70 (32 H), especially 1.47, 1.13 (2s, 3H each, α and β C6 CH_3's), 1.21 (s, 6H, gem di-CH_3's), and 0.83 ppm (t, 3H, ω-CH_3); mass spectrum m/e, 372 (M^+).

RESULTS

A total of 362 microorganisms were tested for their ability to modify nabilone. These cultures represented 206 actinomycetes, 113 molds, and 43 bacterial isolates. About 44% of all of the microorganisms tested produced at least one transformation product, as determined by thin-layer chromatography. A transformation product was defined as a new spot on the chromatogram that absorbed UV light, reacted with fast blue B spray, and was not present in control cultures.

About 60% of the cultures that transformed nabilone appeared to produce compound 2a and/or compound 2b, usually together with other products. The ability to modify nabilone was more prevalent among the actinomycete cultures (~50% produced transformation products) than among the molds (~30%) or bacteria (~20%).

The products isolated from three cultures are shown in Fig. 1. The yields of all of the transformation products were low. An estimation of yields, based on relative UV absorption on thin-layer chromatography plates and overall recovered weight of products, indicated that all products (except compounds 2a and 2b) were produced in yields of 2 to 5% (wt/wt), based on the amount of nabilone added to the cultures. Products 2a and 2b were produced in yields as high as 50% by some microorganisms.

Hydroxylated product 2a was isolated from culture A24007. Spectral data for this product were identical in all respects to the data for an authentic sample of compound 2a (D. W. Johnson and R. A. Archer, Abstr. Joint Central-Great Lakes Reg. Meet. Am. Chem. Soc., 24–26 May 1978, p. 123), which was prepared by a stereoselective reduction of optically active compound 1a (4). Notice that the absolute stereochemistry at position 9 is S, whereas the 6a and 10a positions are both R. Thus, the 6aR,10aR optical isomer (compound 1a) of nabilone (compound 1) was stereospecifically reduced by culture A24007 to the 9S alcohol (compound 2a). A similar 9-hydroxy product was isolated from a culture of *B. cereus* NRRL B8172. In this case, the spectral data were identical to those obtained from an authentic sample of compound 2b, prepared by the stereoselective reduction of optically active compound 1b (4). Again, the microorganism stereospecifically reduced the 9-keto group of compound 1b to the 9S alcohol. The enantiomer of the reduced product (i.e., 6aR,10aR,9R) was not detected.

Four additional products were isolated from *B. cereus* cultures. The assignment of structures to these products is based on interpretations and comparisons of spectral data. In the case of compound 3a, the empirical formula $C_{24}H_{38}O_4$ was obtained from a high-resolution mass spectrum which exhibited a molecular ion at m/e 390.2769. This empirical formula indicates that one oxygen and two hydrogens have been added to nabilone (compound 1). The absence of a carbonyl group in the IR spectrum (5.88 μm, C=O) of compound 3a, coupled with the similarity of the PMR spectrum of compound 3a to that of compound 2a, indicated that the 9-keto group of compound 1 was reduced to a 9-axial hydroxyl group (Table 1). The assignment of the axial orientation is based upon the appearance of a resonance at δ 4.28 ppm, which has been assigned to the 9-equatorial hydrogen of compound 2a as compared with the 9-axial hydrogen of compound 2b. The optical rotation ($[\alpha]_D^{25}$ = 59.9°), coupled with a negative circular dichroism (CD) curve, establishes the chirality of 10a

TABLE 1. *PMR data for nabilone (compound 1) and microbial conversion products*[a]

Resonance	Compound								
	1	2a	2b	3a	4a	5b	6b	7	8
H_4	6.36d	6.36d	6.34d	6.34d	6.34d	6.34s	6.34d	6.44d	6.40d
H_2	6.34d	6.28d	6.19d	6.31d	6.28d		6.30d	6.33d	6.28d
H_9		4.28brs	4.04-3.67m	4.28brs	4.29brs				
$H_{10\alpha}$	4.15d	3.22brd	3.53brd	2.96brd	3.27brd	4.08brd	4.08brd	3.82brd	3.82brd
H_{10a}								2.83m	2.83m
6β-CH_3	1.47s	1.36s	1.37s	1.37s	1.37s	1.47s	1.47s	1.43s	1.42s
6α-CH_3	1.13s	1.03s	1.05s	1.06s	1.06s	1.11s	1.11s	1.08s	1.08s
1'-CH_3's	1.20s	1.18s	1.18s	1.19s	1.19s	1.20s	1.20s	1.35s	1.20s
5'-H's					2.37t		2.35t		
H_6'				3.74m		3.74m			
7'-H's	0.83t	0.83t	0.83t	1.15d	2.08s	1.15d	2.07s		
CH_2-COOH								2.53brs	2.44m

[a] The table reports shift values (σ) measured in a $CDCl_3$ solution (except for compounds 7 and 8, which were measured in acetone-d_6 solution) with tetramethylsilane as an internal reference. Abbreviations: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

as R (A. D. Kossoy, R. A. Archer, D. W. Johnson, and D. E. Dorman, Abstr. Joint Central-Great Lakes Reg. Meet. Am. Chem. Soc., 24-26 May 1978, p. 115). Since the stereochemistry of the 6a,10a ring juncture is *trans*, position 6a must also have the R absolute chirality. Finally, position 9 must, therefore, have the S absolute chirality to be consistent with the hydroxyl group being axial. Thus, the absolute stereochemistry of compound 3a is 6aR,10aR,9S.

Hydroxylation of compound 2a must have occurred at the 6' position of the 1,1-dimethylheptyl side chain to give compound 3a. The position of the side chain hydroxylation is verified by the disappearance in the PMR spectrum of compound 3a of the triplet assigned to the terminal CH_3 group (0.88 ppm) and the appearance of a doublet at δ 1.15 ppm (J = 6 Hz). Such a change in PMR spectra was previously used to assign similar side chain hydroxylation positions for Δ^8-THC (3) and $\Delta^{6a,10a}$-THC (6). The absolute chirality of position 6' in compound 3a has not been established.

Another product (compound 4a) obtained from incubation of compound 1 with *B. cereus* NRRL B8172 had an empirical formula of $C_{24}H_{36}O_4$, indicating that one oxygen atom was added to nabilone. The structure of compound 4a was determined by a comparison of PMR data for compound 4a with those for other model compounds, especially compounds 1, 2a, and 2b. Notice (Table 1) that compound 4a has a pattern of chemical shifts for H_9, $H_{10\alpha}$, 6β-CH_3, and 6α-CH_3 that is consistent with the chemical shifts for axial 9-hydroxy compounds 2a and 3a and inconsistent with either 9-equatorial hydroxy (compound 2b) or 9-keto (compound 1) structure. CD measurements of compound 4a gave a negative CD curve, which has previously been correlated with the 10aR absolute chirality. Therefore, like compound 3a, compound 4a is assigned the 6aR,10aR,9S absolute stereochemistry. With regard to the additional oxygen of compound 3a, notice that the PMR spectrum of compound 3a contains neither a triplet at 0.83 ppm nor a doublet at 1.15 ppm, but instead has a singlet at 2.08 ppm. The chemical shift of this three-proton singlet is consistent with a methyl group next to a ketone group. Furthermore, a two-proton triplet at 2.37 ppm is assigned to the C5' protons next to the C6' ketone. Thus, compound 4a is an optically active 9S-hydroxy-6'-keto-6a,10-*trans* cannabinoid.

An exact mass determination for the molecular ion of compound 5b showed that it was identical in molecular weight and empirical formula to compound 4a. Again, PMR spectral data provided the key to structural assignment. A comparison of the PMR spectrum of compound 5b with the spectra of compounds 1, 2a, 2b, 3a, and 4a showed that compound 5b contained signals for H_{10a}, 6β-CH_3, and 6α-CH_3 which are consistent with a 6a,10a-*trans*-9-keto structure similar to that of compound 1. The absolute chirality of 10a was assigned to S because of the negative CD curve. The similarity of PMR spectral patterns for the side chain protons of compounds 3a and 5b established that compound 5b is also a 6'-hydroxy cannabinoid (especially notice the quartet at 3.74 ppm in the spectra of both compound 3a and compound 5b, which is assigned to the protons at C6', and the doublet at 1.15 ppm assigned to the terminal methyl group). Thus, compound 5b is a 6aS,10aS-9-keto-6'-hydroxy cannabinoid.

Comparison of the PMR spectrum of compound 6b with the spectra of compounds 1, 2a, 2b, 3a, 4a, and 5b showed that compound 6b

contained the $H_{10\alpha}$ signal at 4.08 ppm, similar to compounds 1 and 5b and characteristic of a 9-keto-6a,10a-*trans* structure. The absolute configuration at 10a was established as S by CD measurements. Finally, signals at 2.35 ppm (5'H's) and 2.07 ppm (7'H's) in the PMR spectrum of compound 6b are similar in chemical shift and multiplicity to signals in the spectrum of compound 4a and thereby establish the 6'-keto portion of the structure. High-resolution measurements on the molecular ion of compound 6b in the mass spectrum were consistent with the empirical formula of the suggested structure. Thus, compound 6b is assigned the 6',9-diketo-6aS,10aS structure shown in Fig. 1.

In contrast to the conversion products obtained with *B. cereus* or bacterium A24007, the products obtained from *N. salmonicolor* contained a terminal carboxyl group on the alkyl side chain (Fig. 1). Exact mass determinations for the molecular ions in the mass spectra of compounds 7 and 8 gave the empirical formulas $C_{20}H_{26}O_5$ and $C_{22}H_{30}O_5$, respectively. A comparison of PMR data (Table 1) for compounds 7 and 8 with those for the starting material, compound 1, established that oxygenation occurs on the alkyl side chain. Notice that signals assigned to H_4, H_2, H_{10}, 6β-CH_3, 6α-CH_3, and 1',1'-CH_3's are nearly identical for compounds 7, 8, and 1 (minor differences in chemical shift are attributed to the solvent differences [$CDCl_3$ versus acetone-d_6]). However, the triplet at 0.83 ppm assigned to the terminal methyl group of compound 1 is absent in the spectra of both compound 7 and compound 8. Furthermore, the spectra of both compound 7 and compound 8 contain a two-proton signal (2.53 ppm in compound 7 and 2.44 ppm in compound 8), which is consistent with a methylene group adjacent to a carboxylic acid group. Titration data in 80% aqueous methanol (pK_a 6.87 for compound 7 and 6.76 for compound 8), coupled with a broad band in the IR spectra of both compound 7 and compound 8, provided further evidence for the presence of a carboxylic acid function at the terminus of the alkyl side chain. Thus, compounds 7 and 8 are assigned to the 9-keto-6a,10a-*trans*-carboxylic acid structures shown in Fig. 1.

A partially purified mixture of acidic products was obtained by high-pressure liquid chromatography of the crude culture extract from *N. salmonicolor*. This mixture was analyzed by combined gas chromatography-mass spectrometry after silylation with bis-trimethylsilyltrifluoroacetamide (Regisil) at 80°C for 0.5 h. Five major components were seen as peaks in the chromatogram. The first component (7.4% of the total by integration of peak areas on the chromatogram) that eluted from the polysilicone column (4 feet; 3.8% W-98 on Chromosorb W; temperature, 215 to 217°C) was shown by mass spectrometry to contain two products with molecular ions at *m/e* 492 and 564. These molecular ions are consistent with the molecular weights of polysilylated derivatives of structures 9a and 9b (*m/e* 492 represents disilylation, and *m/e* 564 represents trisilylation).

The second component to elute (22.1%) also showed molecular ions in the mass spectrum at *m/e* 492 and 564. These results are consistent with the diastereomeric structures 9a and 9b. We suggest that compounds 9a and 9b contain a 9S-hydroxy group similar to products 2a, 2b, 3a, and 4a. This 9S configuration results from the stereospecific reduction of the 9-ketone group in compound 1.

The third component (11.2%) to elute from the column gave rise to molecular ions at *m/e* 490, 564, and 592. The product represented by *m/e* 490 is presumed to be, by virtue of its mass spectral fragmentation pattern, the disilylated derivative of compound 7. Although the presence of a molecular ion at *m/e* 564 could indicate a 9R-hydroxy diastereomer of compound 9a or 9b, which in turn would suggest that stereospecific reductions of the 9-ketone group of compound 1 did not occur, we believe that the appearance of the *m/e* 564 ion in this region is indicative of the "tailing" of the previous peak into this region. The molecular ion at *m/e* 592 is derived from the trisilylated derivative of either compound 10a or compound 10b.

The fourth component (56.2%) to elute appeared as a single peak with a molecular ion at *m/e* 592. This molecular weight is consistent with trisilylated compound 10a or 10b, depending upon the chiralities at positions 6a, 10a, and 9. As previously observed with *B. cereus* or culture A24007, reduction of the 9-ketone group of compound 1 is stereospecific and yields 9S-hydroxy products. Therefore, compounds 10a and 10b are tentatively assigned the 6aR,10aR,9S and 6aS,10aS,9S structures, respectively.

The fifth broad peak (4.1%) gave rise to a molecular ion at *m/e* 620. This, together with the fragmentation data, is consistent with a structural assignment for the compound as the trisilylated derivative of compound 11a or 11b.

In summary, the structural assignments for compounds 2a and 2b are based upon comparison of spectral data with authentic samples. The assignments of compounds 3a, 4a, 5b, 6b, 7, and 8 are based upon comparisons and interpretations of spectral data, especially PMR data (Table 1). And the structural assignments for compounds 9a, 9b, 10a, 10b, 11a, and 11b are based primarily upon the consistency of the gas chro-

matography-mass spectrometry data with the expected trimethylsilane derivatives of the suggested structures.

Summary of physical and chemical data. (i) (−)-*trans*-3-(1',1'-Dimethylheptyl)-6,6aR,7,8,10,10aR-hexahydro-1,6',9S-trihydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran (compound 3a). UV (ethanol) λ_{max}, 208, 225 (shoulder), and 275 nm (ϵ = 32,000, 7,300, and 130); IR ($CHCl_3$), 2.98 μm (OH); ^1H NMR ($CDCl_3$), 6.34, 6.31 (2d, 1H each, J = 2 Hz, H_2 and H_4), 4.28 (broad s, 1H, H_9 equatorial), 3.02-1.02 (33 H), especially 2.96 (broad s, 1H, H_{10a}), 1.37 (s, 3H, 6β-CH_3), 1.19 (s, 6H, gem di-CH_3's), 1.15 (d, 3H, J = 6 Hz, CH_3CH=OH), and 1.06 ppm (s, 3H, 6α-CH_3); an exact mass determination gave *m/e* 390.2769 (calculated value for $C_{24}H_{38}O_4$, 390.2770); $[\alpha]_D^{25} = -59.9°$ (C4, ethanol).

(ii) (−)-*trans*-3-(1',1'-Dimethylheptyl)-6-6aR,7,8,9,10,10aR-hexahydro-1,9S-dihydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran-6'-one (compound 4a). UV (ethanol) λ_{max}, 208, 227 (shoulder), and 280 nm (ϵ = 19,000, 4,600, and 80); IR ($CHCl_3$), 2.98 (OH) and 5.88 μm (C=O); ^1H NMR ($CDCl_3$), 6.34, 6.28 (2d, 1H each, J = 2 Hz, H_2 and H_4), 4.29 (broad s, 1H, H_9 equatorial), 3.27 (broad d, 1H, J = 15 Hz, H_{10} equatorial), 3.0-1.02 (32 H), especially 2.96 (broad s, 1H, H_{10}), 2.37 (t, 2H, CH_2-C=O), 2.08 (s, 3H, CH_3C=O), 1.37 (s, 3H, 6β-CH_3), 1.19 (s, 6H, gem di-CH_3's), and 1.06 ppm (s, 3H, 6α-CH_3); an exact mass determination gave *m/e* 388.2614 (calculated value for $C_{24}H_{36}O_4$, 388.2613); $[\alpha]_D^{25} = -18.6°$ (Cl, ethanol).

(iii) (+)-*trans*-3-(1',1'-Dimethylheptyl)-6,6aS,7,8,10,10aS-hexahydro-1,6'dihydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran-9-one (compound 5b). UV (ethanol) λ_{max}, 207, 225 (shoulder), and 280 nm (ϵ = 16,800, 4,200, and 80); IR ($CHCl_3$), 3.03 (OH) and 5.90 μm (C=O); ^1H NMR ($CDCl_3$), 6.34 (s, 2H, H_2 and H_4), 4.08 (broad d, 1H, J = 14 Hz, H_{10a}), 3.74 (m, 1H, $H_{6'}$), 3.02-1.02 (32 H), especially 1.47 (s, 3H, 6β-CH_3), 1.20 (s, 6H, gem di-CH_3's), 1.15 (d, 3H, J = 6 Hz, CH_3C-OH), and 1.11 ppm (s, 3H, 6α-CH_3); $[\alpha]_D^{25} = +46.3°$ (C3, $CHCl_3$); an exact mass determination gave *m/e* 388.2614 (calculated value for $C_{24}H_{36}O_4$, 388.2613).

(iv) (+)-*trans*-3-(1',1'-Dimethylheptyl)-6,6aS,7,8,10,10aS-hexahydro-1-hydroxy-6,6-dimethyl-9H-dibenzo[b,d]pyran-6',9-dione (compound 6b). UV (ethanol) λ_{max}, 208, 230 (shoulder), and 280 nm (ϵ = 38,000, 10,000, and 200); IR ($CHCl_3$), 3.04 (OH) and 5.88 μm (C=O); ^1H NMR ($CDCl_3$), 6.77 (s, 1H, exchanges with D_2O), 6.34, 6.30 (2d, 1H each, J = 2 Hz, H_2 and H_4), 4.08 (broad d, 1H, J = 14 Hz, H_{10}), 3.03-1.02 (30 H), especially 2.35 (t, 2H, J = 7 Hz, $H_{5'}$), 2.07 (s, 3H, CH_3C=O), 1.47 (s, 3H, 6β-CH_3), 1.20 (s, 6H, gem diCH_3's), and 1.11 ppm (s, 3H, 6α-CH_3); an exact mass determination gave *m/e* 386.2461 (calculated value for $C_{24}H_{34}O_4$, 386.2457).

(v) 6a,7,8,9,10,10a-Hexahydro-1-hydroxy-1,1,6,6-tetramethyl-9-oxo-6H-dibenzo[b,d]pyran-3-propanoic acid (compound 7). IR (KBr), 3,200, 1,705, 1,620, 1,580, and 1,420 cm^{-1}; pK_a (80% methanol) 6.87; ^1H NMR (acetone-d_6)δ 6.44, 6.33 (2d, 1H each, J = 2 Hz, H_4 and H_2), 3.82 (broad d, 1H, J = 14 Hz, H_{10} equatorial), 3.0-1.08 (23 H), especially 2.83 (m, 1H, H_{10a}), 2.53 (broad t, 2H, CH_2-COOH), 1.43 (s, 3H, 6β-CH_3), 1.35 (s, 6H, gem di-CH_3's), and 1.08 (s, 3H, 6α-CH_3); an exact mass determination gave *m/e* 346.1781 (calculated value for $C_{20}H_{26}O_5$, 346.1780).

(vi) 6a,7,8,9,10,10a-Hexahydro-1-hydroxy-1,1,6,6-tetramethyl-9-oxo-6H-dibenzo[d,b]pyran-3-pentanoic acid (compound 8). IR (KBr), 3,320, 3,180, 1,740, 1,720, 1,700, 1,630, 1,585, and 1,420 cm^{-1}; pK_a (80% methanol) 6.76; ^1H NMR (acetone-d_6)δ 6.40, 6.28 (2d, 1H each, J = 2 Hz, H_2 and H_4), 3.82 (dd, 1H, J = 14, 3 Hz, H_{10a}), 3.0-1.08 (27 H), especially 2.83 (m, 1H, H_{10}), 2.44 (broad t, 2H, $-CH_2$-COOH), 1.42 (s, 3H, 6β-CH_3), 1.20 (s, 6H, gem di-CH_3's), and 1.08 (s, 3H, 6α-CH_3); an exact mass determination gave *m/e* 374.2093 (calculated value for $C_{22}H_{30}O_5$, 374.2092).

DISCUSSION

Nabilone (compound 1) is transformed by *B. cereus* NRRL B8172 into several oxidized products (compounds 2b, 3a, 4a, 5b, and 6b). In each of these products, oxygenation of the 1,1-dimethylheptyl side chain occurred on the carbon atom adjacent to the terminal methyl group. One of these products, compound 3a, appears to be identical to a human metabolite of nabilone (19). Significantly, all of the products are optically active, although the substrate, nabilone, is optically inactive. In the cases of compounds 2a, 2b, 3a, and 4a, a new center of asymmetry was introduced by microbial reduction of the C9 ketone of compound 1. With each of these products, evidence was obtained that microbial reduction of compound 1 occurs sterospecifically to 9S-hydroxy isomers. When microbial reduction of the C9 ketone did not occur (as in compound 6b), an optically active product also resulted, indicating that the side chain-oxidizing enzyme selectively attacks the 6aS,10aS isomer of compound 1. If this were not the case, compound 6b would be expected to be optically inactive. Our results also suggest that compounds 3a and 4a are initially transformed into their 9S-hydroxy metabolites and that it is the

6aR,10aR ring juncture isomers that undergo further modification.

N. salmonicolor ATCC 19149 oxidized the 1,1-dimethylheptyl side chain of compound 1 to carboxylic acids of varying chain length. The microbial oxidation of compound 1 by *N. salmonicolor* ATCC 19149 leads to two series of carboxylic acids: one containing a carbonyl group at C9 and terminal carboxylic acid side chains of 5, 7, and 9 carbons (see structures 7 and 8), and the other having presumably a 9S hydroxyl group with terminal carboxylic acid side chains of 5, 7, and 9 carbon atoms (Fig. 1, structures 9a, 9b, 10a, 10b, 11a, and 11b). The acid derivatives, compounds 8, 9a, 9b, 10a, and 10b, require a shortening of the 1,1-dimethylheptyl side chain of compound 1 by two or four carbon atoms. Such products probably arise via a terminal oxidation–beta-oxidation mechanism similar to that reported for the microbial oxidation of alkanes (21).

The results obtained from this screening program indicate that many microorganisms, approximately 44% of those tested, are capable of modifying the synthetic cannabinoid nabilone. With the exception of the 9-hydroxy derivatives, compounds 2a and 2b, the yields of the products were quite low. The use of a nabilone-polyvinylpyrrolidone codispersion as the substrate resulted in somewhat higher product yields, presumably due to better dispersion of nabilone in the culture medium. Although no other attempts were made to increase yields, a study of fermentation variables could lead, as has been the case with steroid transformations, to yield improvement.

Many transformation products of nabilone detected in the screening program have not yet been isolated or identified. In view of the unique transformation products that were isolated and identified, microbial modification may be considered a versatile tool for obtaining a variety of new cannabinoids. Some of these new microbial products may have desirable pharmacological properties or may be useful as intermediates for further chemical modification.

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